RESEARCH NOTE

TETRAZOLIUM TEST FOR SEED VIABILITY AND GERMINABILITY OF *MELOCACTUS ERNESTII* VAUPEL SUBSP. *ERNESTII* AND *MELOCACTUS ZEHNTNERI* (BRITTON & ROSE) LUETZELB. (CACTACEAE)

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ABSTRACT – Despite its ornamental value, some *Melocactus* species (Cactaceae) are threatened by several factors and only sexual propagation is possible. Thus, artificial seed banks are an appropriate method for their *ex situ* conservation. Suitable methods to germinate and to monitor viability are necessary for the seeds of these species. This work explored the use of tetrazolium test for monitoring seed viability of two *Melocactus* species. There was a correlation between the percentage of stained embryos, considering different cover area or tone stain, and the germination percentage. Unviable embryos did not stain. The embryos curved shape can explain the partial stain on the most of cases.
KEY WORDS: *germplasm bank, germination, ex situ conservation.*

TESTE DE TETRAZÓLIO PARA AVALIAR A VIABILIDADE DE SEMENTES E GERMINABILIDADE DE *MELOCACTUS ERNESTII* VAUPEL SUBSP. *ERNESTII* E *MELOCACTUS ZEHNTNERI* (BRITTON & ROSE) LUETZELB. (CACTACEAE)

RESUMO – Apesar de seu valor ornamental, algumas espécies de *Melocactus* (Cactaceae) estão ameaçadas por diversos fatores e apenas a propagação sexual é possível. Assim, os bancos de sementes artificiais são apropriados para sua conservação *ex situ*. São necessários, entretanto, métodos para monitorar a germinabilidade e viabilidade das sementes destas espécies. Esse trabalho explorou o uso do teste do tetrazólio para monitorar a viabilidade das sementes de duas espécies de *Melocactus*. Houve uma correlação entre a porcentagem de embriões corados, considerando diferentes áreas de cobertura e tonalidade de coloração, e a porcentagem de germinação.
PALAVRAS-CHAVE: *bancos de germoplasma, germinação, conservação ex situ*.

PRUEBA DE TETRAZOLIO PARA EVALUAR LA VIABILIDAD DE LAS SEMILLAS Y LA GERMINABILIDAD DE *MELOCACTUS ERNESTII* VAUPEL SUBSP. *ERNESTII* E *MELOCACTUS ZEHNTNERI* (BRITTON & ROSE) LUETZELB. (CACTACEAE)

RESUMEN – A pesar de su valor ornamental, algunas especies de *Melocactus* (Cactaceae) están amenazadas por diversos factores y solamente la propagación sexual es posible. Así, los bancos de semillas artificiales son adecuados para su conservación *ex situ*. Sin embargo, son necesarios métodos para monitorizar la germinabilidad y la viabilidad de las semillas de estas especies. Esa investigación ha explorado la utilización de la prueba de tetrazolio para evaluar la viabilidad de las semillas de dos especies de *Melocactus*. Se observó una correlación entre el porcentaje de embriones coloreados, teniendo en cuenta las diferentes áreas de cobertura y el tono de color, y el porcentaje de germinación.
PALABRAS CLAVE: bancos de germoplasma, germinación, conservación *ex situ*.

INTRODUCTION

The genus *Melocactus* Link & Otto belongs to Cactoideae subfamily and comprises 31 species distributed in South and Central America. Most of the species grow in Bahia and north of Minas Gerais states (Brazil). Therefore, this area is considered the diversity primary center of the genus, where 14 species, most of the endemics, are found (Taylor, 1991). Some *Melocactus* species are threatened by several factors: degradation of habitats because of building pressure on the coastal region; the vegetation firing of Caatinga vegetation, their natural biome; and illegal gathering for trade as ornamental plants (BDT, 2000; CITES, 2007). Furthermore, the propagation of *Melocactus* species in nature is possible just by seeds.

There are few efforts to create cactus germplasm banks and the optimum conditions for long term storage is not known for most cactus species according Rojas-Aréchiga and Vázquez-Yanes (2000). Hong *et al.* (1998) describe the response to desiccation on silica gel, cold storage and define the optimum conditions for *Opuntia* Mill. and *Ferocactus* Britton & Rose seed germination and conservation. According to Ocampo-López (2003), the seed viability of *Mammillaria supertexta* Mart. ex Pfeiff. is maintained for several years if store under the appropriate conditions. Work has also been carried out on *in vivo* or *in vitro* conservation strategies (Maiti *et al.*, 2002; Giusti *et al.,* 2002; Moebius-Goldammer *et al.,* 2003; Ramirez-Malagon *et al.,* 2007). Knowledge is needed on the seed biology of these species for the successful establishment of seed banks. Appropriate methods to germinate and to monitor seed viability are necessary.

MATERIAL AND METHODS

We explored the use of the tetrazolium test for monitoring *Melocactus* seed viability. Seeds of two species packed in paper bags were studied: *Melocactus ernestii* Vaupel subsp. *ernestii* seeds that had been stored for more than three years at temperature room (25-32°C) and *Melocactus zehntneri*

(Britton & Rose) Luetzelb. seeds either stored in refrigerator (5°C, for 34 months collected 2-3 months before the test). New collected seeds of the two species were also tested.

The embryos were extracted after soaking the seeds in water for 24 h. After removal of the embryo, they were submitted to a 0.6% tetrazolium solution overnight at 30°C (ISTA, 2003). The embryos were observed under a stereo microscope and assorted according to the percentage of stained area and color tone (red or rose). These data were used to establish viability criterions of *Melocactus* seeds.

Germination of *Melocactus* seeds were tested under a constant temperature of 25°C with a 16-h light photoperiod (provided by cool white fluorescent tubes with an irradiance of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Four replicates of 25 seeds each were tested for germination on top of two sheets of filter paper (previously moistened with 3.5 ml distilled water) in 7-cm diameter glass Petri dishes. Filter papers were rewetted regularly with distilled water as required. Dishes were checked three times a week over a total 30-day test period and germinated seeds (germination was determined as radical protrusion) were counted and removed.

RESULTS AND DISCUSSION

No germination was observed on *M. ernestii* subsp. *ernestii* seeds stored on a determined temperature room (annual media of 25,5°C) during 36 months, neither on the *M. zehntneri* seeds stored on refrigerator for 34 months. Similarly, no embryos of these seeds were stained with tetrazolium. Similar results were obtained by Salles (1987) using *Coleocephalocereus fluminensis* (Miq.) Backeb. seeds at least 6 years old.

On the other hand, the percentage of stained embryos of the new collected *M. zehntneri* seeds was 87%. The table 1 shows the intensity and area stained criterions. Some embryos were partially stained varying the red stained area **(Table 1)**. According to ISTA rules (ISTA, 1991), for seeds without endosperm, a complete and uniform radicle and plumule stain is necessary.

TABLE 1. Viability percentage of *Melocactus zehntneri* (Britton & Rose) Luetzelb. (Cactaceae) seeds according criterions of stain (colors red or rose) obtained tetrazolium test.

	Embryo area stained			
Color of stain	≥ 75%	≥ 50%	≥ 25%	≥ 5%
Red	4%	21%	46%	58%
Rose	0%	0%	13%	29%
Viable seeds	**4%**	**21%**	**59%**	**87%**

Considering that the seed germination of *M. zehntneri* reached 92%, we feel confident that all the embryos that stained, although partially, were viable. The embryos curved shape and manipulation damages removing the testa could account for the partial stain on the most of cases.

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